possible that the observed reduced proteasomal activity is secondary to the severe neuronal loss in the SN. In another study, Furukawa et al. [3] found preserved proteasome peptidase activities in the striatum and cortices of PD patients, patients with multiple system atrophy, and those with progressive supranuclear palsy. Preservation of proteasome peptidase activities in the striatum and decrement in the SN suggest the presence of fragile mechanisms to systematic stresses in the SN. Indeed, the immunohistochemical findings of the proteasome subunits were similar in both the striatum and SN, suggesting vulnerability of proteasome activities in the SN of PD.

In conclusion, we have demonstrated immunohistochemically in the present study the distribution of the 20S proteasome in the nuclei of neurons in PD. Further studies are needed to understand the mechanism responsible for the localization of nuclear proteasome.

Acknowledgments

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Decline of Striatal Dopamine Release in Parkin-Deficient Mice Shown by Ex Vivo Autoradiography

Shigeto Sato,[1,2] Tomoki Chiba,[2] Shingo Nishiyama,[3] Takeharu Kakiuchi,[3] Hideo Tsukada,[3] Taku Hatano,[1] Takahiro Fukuda,[4] Yasunobu Yasoshima,[5] Nobuyuki Kai,[5] Kazuto Kobayashi,[5] Yoshikuni Mizuno,[1] Keiji Tanaka,[2] and Nobutaka Hattori[1]*

[1]Department of Neurology, Juntendo University School of Medicine, Bunkyo, Tokyo, Japan
[2]Tokyo Metropolitan Institute of Medical Science, Bunkyo, Japan
[3]Central Research Laboratory, Hamamatsu Photonics K.K., Hamamatsu, Shizuoka, Japan
[4]Division of Neuropathology, Department of Neuroscience, Research Center for Medical Sciences, The Jikei University School of Medicine, Minato-ku, Tokyo, Japan
[5]Department of Molecular Genetics, Institute of Biomedical Sciences, School of Medicine, Fukushima Medical University, Fukushima, Japan

Parkin is the causal gene of autosomal recessive juvenile parkinsonism (AR-JP). Dopamine (DA) metabolism has been linked to Parkinson's disease (PD). To understand the pathogenesis of AR-JP, we generated parkin-deficient mice to assess the status of DA signaling pathway and examine DA release and DA receptor by ex vivo autoradiography. Ex vivo autoradiography using [^{11}C]raclopride showed a clear decrease in endogenous DA release after methamphetamine challenge in parkin-deficient mice. Furthermore, parkin deficiency was associated with considerable upregulation of DA (D_1 and D_2) receptor binding in vivo in the striatum and increased DA levels in the midbrain. Our results suggest that dopaminergic neurons could behave abnormally before neuronal death.

Key words: parkin; dopamine; release; autoradiography; receptor

Parkinson's disease (PD) is the most common neurodegenerative movement disorder in the elderly and affects approximately 1% of the population >65 years of age. The major symptoms of PD are tremor, bradykinesia, cogwheel rigidity, and postural instability, which arise from the degeneration of dopaminergic (DAergic) neurons in the substantia nigra (SN). Of the many hereditary PD genes, the *parkin* gene has received special interest by researchers working in the field of PD (Kitada et al., 1998), because it displays ubiquitin-ligase activity and that early investigations showed that proteolytic dysfunction causes massive loss of DAergic neurons (Shimura et al., 2000; Chung et al., 2001; Imai et al., 2001). Several lines of parkin-null mice have been generated, however, the mechanism(s) underlying the cause of autosomal-recessive-juvenile-parkinsonism (AR-JP) are less well defined, because most (if not all) substrates for parkin reported so far remain unchanged irrespective of parkin-deficiency (our unpublished results) (Goldberg et al., 2003; Periquet et al., 2005). The aim of the present study was to uncover the pathogenesis of AR-JP using parkin-deficient mice.

Imaging of changes in neuroreceptor availability to positron emission tomography (PET) ligands can be used to indirectly measure synaptic neurotransmitter fluxes in the living human brain and several PET studies in PD have been reported (Dagher, 2001; de la Fuente-Fernandez and Stoessl, 2002). In PD, the characteristic loss of striatal dopamine (DA) terminal function, reflected by reduced dopa decarboxylase activity, can be quantified in vivo using [^{18}F]dopa PET. Although striatal [^{18}F]dopa uptake reflects the storage of DA, [^{11}C]raclopride, an in vivo marker of dopamine D_2 receptor, is useful for assessing the capacity of endogenous DA release (Piccini et al., 2003). We monitored the status of DA metabolism in the brains of parkin-null mice, and focused on DA release and DA receptor by using ex vivo autoradiography techniques.

MATERIALS AND METHODS

Generation of Parkin$^{-/-}$ Mice

For the constructed targeting vector, most of exon 2 was replaced in-frame by the coding sequence of τ-GFP to

*Correspondence to: Nobutaka Hattori, MD, PhD, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo, Tokyo, Japan. E-mail: nhattori@med.juntendo.ac.jp

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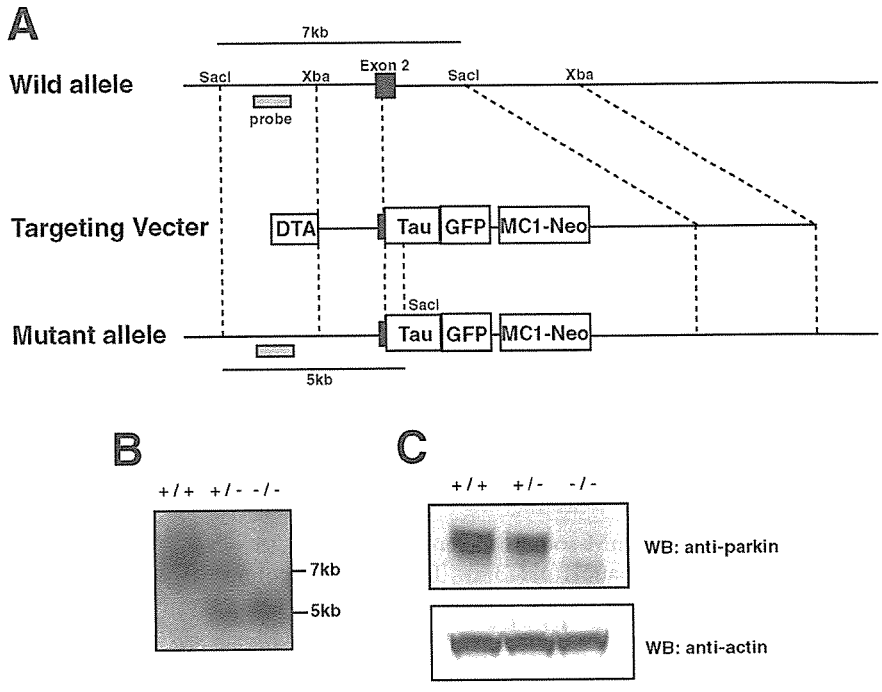


Fig. 1. Generation of parkin-deficient mice. **A:** Schematic representation of the targeting vector and the targeted allele of *parkin* gene. Exon 2 is depicted by the black box. The τ, GFP, MC1-Neo (neomycin-resistant) gene cassette is shown. The probe for Southern blot analysis is shown as a gray square. DTA, diphtheria toxin gene. **B:** Southern blot analysis of genomic DNAs extracted from tails of wild-type (+/+), heterozygous (+/−) or homozygous (−/−) mice. The genomic DNAs digested with *Sac*I were hybridized with the probe shown in (A). Wild-type and mutant alleles are detected as 7- and 5-kb bands, respectively. **C:** Western blotting of parkin in whole brain lysates. The lysates of mice indicated genotypes were immunoblotted with antibodies against parkin and actin.

use as a reporter system for axonal extension, followed by translation and transcription termination sequences and the MC1-neo cassette (Fig. 1A). A targeting vector was constructed using 1.5- and 7-kb DNA fragments as 5′ and 3′ homologous sequences, respectively. A negative selection cassette, DTA, which encodes the diphtheria toxin, was also included. The linearized targeting vector was transfected into TT2 ES cells. After selection in G418, clones were screened by Southern analysis for homologous recombination. Using the 5′ external probe and probe specific for Neo sequence, we confirmed the clones carried the desired homologous recombination. ES cells of clones were injected into C57BL/6J. Chimeric offspring were crossed with C57BL/6J mice to obtain germline transmission, which was confirmed by Southern analysis with the 5′ probe. Heterozygous mice were then interbred to obtain homozygous knockout and wild-type control mice. Mice were subsequently genotyped by PCR using primers specific for the wild-type and the targeted allele.

Behavioral Test

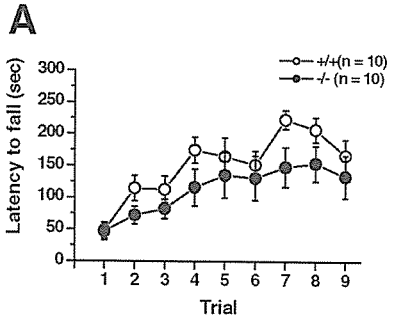
We used the rotorod test in this study, which is a test used commonly to score the severity of motor impairment in rodents. Mice were placed on an accelerating rotorod (ENV-575M, Med Associates, Inc., St. Albans, VT) and the time that a mouse stayed on the rotating drum was recorded. Two behavioral patterns noted on loss of balance on the drum were recorded: falling off the rotating rod and clinging to the rotorod with complete "passive" ride around the rod (Paylor et al., 1999). In the latter behavior, the mouse either continued to walk when it reached the top of the rod, or clung around the rod a second time. Behaviors were divided operationally into two categories. For active performing mice, they never passively clung around the rotorod, whereas mice that clung around the rod at least one time during each trial were defined as passive performing mice. For the active mice, the latency to fall was recorded for each trial. For the passive mice, the latency to fall off the rotorod, or the latency to the first cling around (latency to cling) was recorded. Passive performing mice were allowed to continue to walk on the rotorod after the first passive rotation. Thus, the data for latency to fall in the passive mice represented the whole time spent on the rotating rod with several passive rotations. Each mouse underwent three trials per a day with a 45-min intertrial interval and these tests were conducted over 3 successive days. A two-way ANOVA [genotype × trial] with repeated measures was used to analyze the latency to fall or to cling.

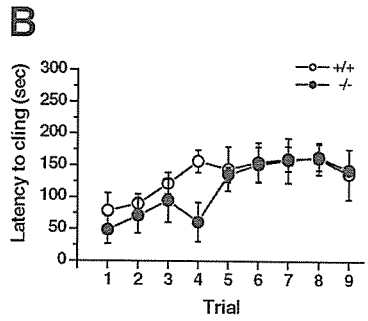
Immunohistochemistry for Tyrosine Hydroxylase

Immunolabeling using anti-tyrosine hydroxylase (TH) antibody (TH-16; dilution 1:10,000; Sigma Biosciences, St. Louis, MO) was detected by the avidin-biotinylated horseradish peroxidase complex method (Vectastain ABC elect kit, Burlingame, CA).

Neurochemical Analysis

Contents of dopamine (DA) and its metabolites 3,4-dihydroxy-phenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by high-performance liquid chromatography (HPLC) equipped with an electrochemical detection system (Kobayashi et al., 1994). Tissues were homogenized in 5 volumes of 0.2 M perchloric acid containing 0.1 M ethylenediaminetetraacetic acid (EDTA) and 100 ng/ml of isoproterenol. After centrifugation of the tissue homogenates, the pH of the supernatants was adjusted to 3.0 by adding 1 M sodium acetate. The samples were injected into the HPLC system with the mobile phase containing 0.1 M sodium citrate,





Number of mice every trial

	Passive		Active	
Trial	+/+	-/-	+/+	-/-
1	2	3	8	7
2	7	4	3	6
3	6	4	4	6
4	7	4	3	6
5	7	3	3	7
6	6	5	4	5
7	8	5	2	5
8	5	3	5	7
9	4	3	6	7

Fig. 2. Results of rotorod motor skill learning test conducted in 12-month-old parkin-deficient and wild-type mice. **A:** Latency to fall. Wild-type (+/+) (n = 10) and knockout (−/−) mice (n = 10) showed equivalent performance and motor learning. The mean ± SEM latency to fall is shown for each trial. **B:** Latency to cling to the rod in passive performing mice. Some mice rode around the rod at least once during training. Number of mice in every trial is tabulated. Active performance, mice that never passively rode around the rotorod (without clinging to the rod); passive performance, mice that rode around the rod at least once during training.

0.1 M citric acid, 0.5 mM sodium octane sulfonate, 0.15 mM EDTA, and 12% methanol (pH 3.5). The detector potential was maintained at 0.75 V vs. the Ag/AgCl electrode.

Ex Vivo Autoradiography

Chemicals. Standard compounds and precursors for labeling of (+)-α-[^{11}C]dihydrotetrabenazine ([^{11}C]DTBZ), [^{11}C]β-CFT (2β-carbomethoxy-3 β-(4-fluorophenyl)tropane: dopamine transporter probe). [^{11}C]SCH23390 (D1 receptor probe) and [^{11}C]raclopride (D_2 receptor probe) were purchased from RBI (Natick, MA). The enzymes for L-[β-^{11}C]DOPA synthesis, alanine racemase (EC 5.1.1.1.), D-amino acid oxidase (EC 1.4.3.3.) and β-tyrosinase (EC 4.1.99.2), were purchased from Ikeda Food Research Co. (Hiroshima, Japan).

Synthesis of [^{11}C]-Labeled Compounds. Carbon-11 (^{11}C) was produced by ^{14}N(p,α)^{11}C nuclear reaction using a cyclotron (HM-18, Sumitomo Heavy Industry, Tokyo) at Hamamatsu Photonics PET center and obtained as [^{11}C]CO_2. [^{11}C]β-CFT and [^{11}C]SCH23390 were labeled with ^{11}C by *N*-methylation of the corresponding nor-compounds with [^{11}C]methyl iodide prepared from [^{11}C]CO_2. [^{11}C]DTBZ and [^{11}C]raclopride were synthesized by *O*-methylation of the corresponding nor-compounds with [^{11}C]methyl iodide. The radiochemical and chemical purities used were >98% and 99%, respectively, and the specific radioactivity ranged from 182–201 GBq/μmol for [^{11}C]DTBZ, 152–181 GBq/μmol for [^{11}C]β-CFT, from 144–162 GBq/μmol for [^{11}C]SCH23390, and from 154–177 GBq/μmol for [^{11}C]raclopride, respectively. L-[β-^{11}C]DOPA (L-3,4-dihydroxyphenylalanine) was synthesized using a combination of organic synthesis and multi-enzymatic procedures using an automated synthesizer. The radiochemical and chemical purities of L-[β-^{11}C]DOPA were >98% and 99%, respectively. After analysis for identification, the solution was passed through a 0.22 μm pore size filter before intravenous (i.v.) administration.

Ex-vivo Imaging. Each labeled compound was injected i.v. at a dose of ca. 1 MBq/g body weight via the tail vein. The animals were sacrificed by decapitation under halothane anesthesia 30 min post-injection for [^{11}C]DTBZ, [^{11}C]SCH23390, and [^{11}C]raclopride, and 60 min post-injection for L-[β-^{11}C]DOPA and [^{11}C]β-CFT. The interval between injection of tracer and sacrifice for each labeled compound was determined based on data from previous reports (Inoue et al., 1991; Tsukada et al., 1994; Takamatsu et al., 2004). The brain was removed immediately, frozen by 2-methyl butane at −20°C, and 2-mm thick brain slices that included the striatal and cerebellar regions were prepared with a brain matrix (RBS-02, Neuroscience Inc., Tokyo, Japan). These slices were contacted with phospho imaging plate for 30 min, and the regional distribution of radioactivity was determined using a phospho imaging plate reader (BAS-1500 MAC, Fuji Film Co., Tokyo, Japan). The radioactivity in the cerebellum was used as the reference because of the low density of DA receptors in this region (Creese et al., 1975). Vesicular monoamine transporter (VMAT) availability, DA reuptake site availability, and DA (D_1 and D_2) receptor binding activities were expressed as follows: "Binding index" = (RIstr − RIcere)/RIcere, where RIstr was the radioactivity of each labeled ligand in the striatal regions and RIcere was the radioactivity in the cerebellum; for the quantification of dopamine synthesis, "Uptake index" was determined as (RIstr − RIcere)/RIcere, where RIstr was the radioactivity of L-[β-^{11}C]DOPA in the striatal regions, and RIcere was the radioactivity in the cerebellum.

Displacement Study of [^{11}C]raclopride With Methamphetamine

To evaluate the dopamine release from pre-synaptic neurons, saline or methamphetamine (MAP) at a dose of 0.3 mg/kg was injected i.v. 30 min before the injection of [^{11}C]raclopride. Brain slice preparation and imaging procedure were carried out as described above.

Statistical Analysis

All data were expressed as mean ± SEM. Differences between groups were examined for statistical significance using the Student's *t*-test. A *P*-value < 0.05 denoted the presence of a statistically significant difference.

RESULTS

Generation of Parkin-Deficient Mice

To investigate the in vivo roles of parkin and shed light on the pathogenic mechanisms of AR-JP, we generated a new line of mice with a targeted disruption of the *parkin* gene. We deleted exon 2 of *parkin* that encodes most of the N-terminal ubiquitin-like domain whose major role is interaction with the 26S proteasome (Sakata et al., 2003). It is noteworthy that several mutations in exon 2 have been reported in patients with AR-JP (Mata et al., 2004). Due to the lack of exon 2, RNA splicing from exons 1–3 is predicted to change the reading frame, which is capable of causing almost complete dysfunction of parkin. We therefore chose the disruption of exon 2 to generate the parkin-null mutant mouse. Deletion of exon 2 was confirmed at the *parkin* genomic locus by Southern analysis (Fig. 1B). Parkin was absent in the knockout brain based on the analysis using anti-parkin antibody (Fig. 1C). Because τ-GFP cDNA was knockin, we also carried out RT-PCR using an GFP-specific primer and confirmed the presence of GFP transcripts in parkin-deficient mice (data not shown). However, the parkin-τ-GFP fusion protein was not clearly detectable by Western and immunohistochemical analyses (data not shown).

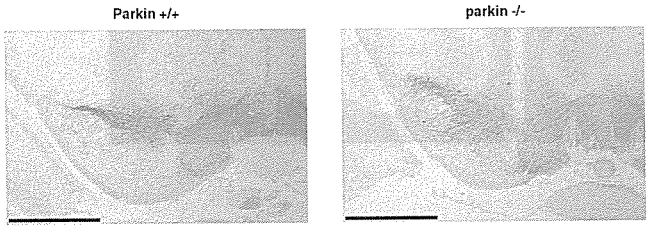


Fig. 3. Immunohistochemical localization of tyrosine hydroxylase (TH) at 3 months in the midbrain and substantia nigra (SN) of parkin-deficient mice and wild-type mice. Normal TH immunoreactivity in the SN. Scale bar = 1 mm.

Phenotype of Parkin$^{-/-}$ Mice

We carried out open field tests of *parkin*$^{-/-}$ mice and observed no significant alterations in their movement behaviors (data not shown). In addition, we eval-

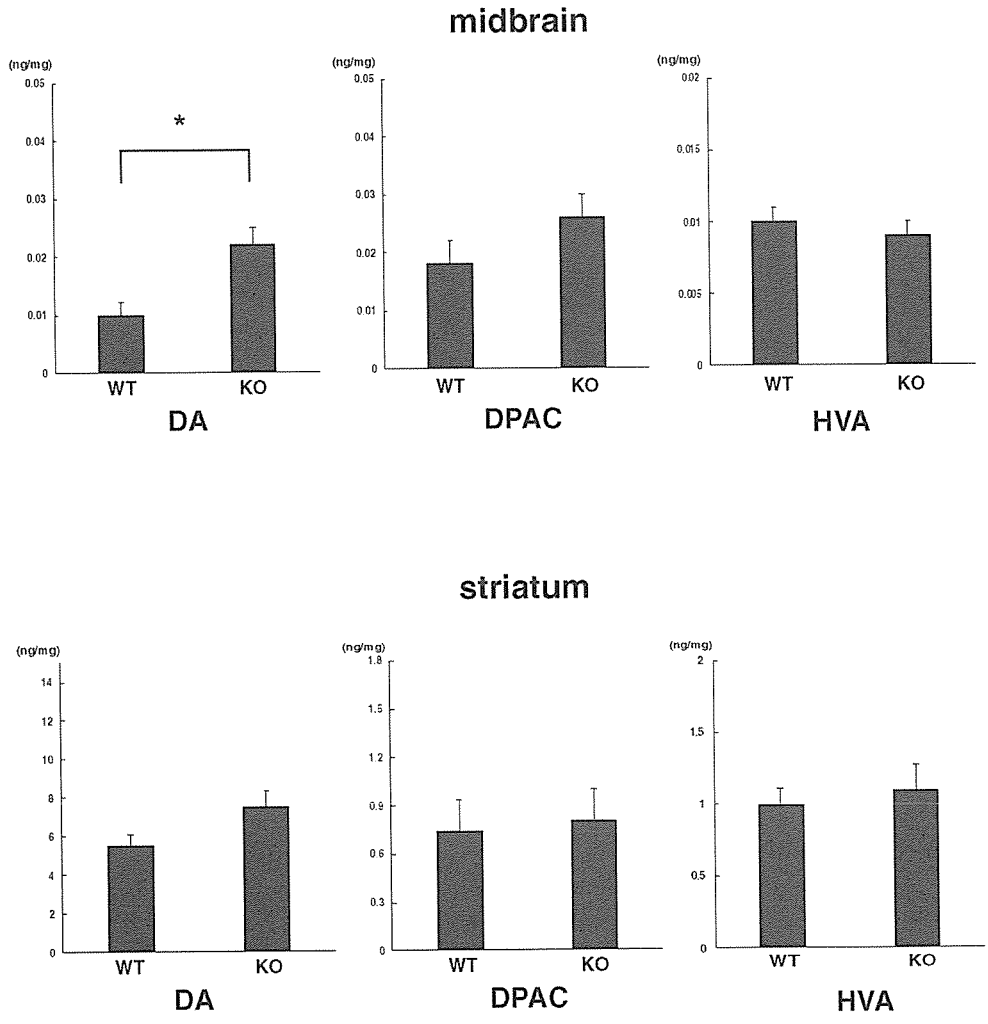


Fig. 4. The levels of DA and its metabolites (DOPAC and HVA) measured at 12 months in the midbrain and striatum in wild-type (WT) (n = 10) and parkin-deficient (KO) mice (n = 10). Concentrations of DA, DOPAC, and HVA were determined by HPLC with electrochemical detection. Data are expressed as mean ± SEM. $*P < 0.01$.

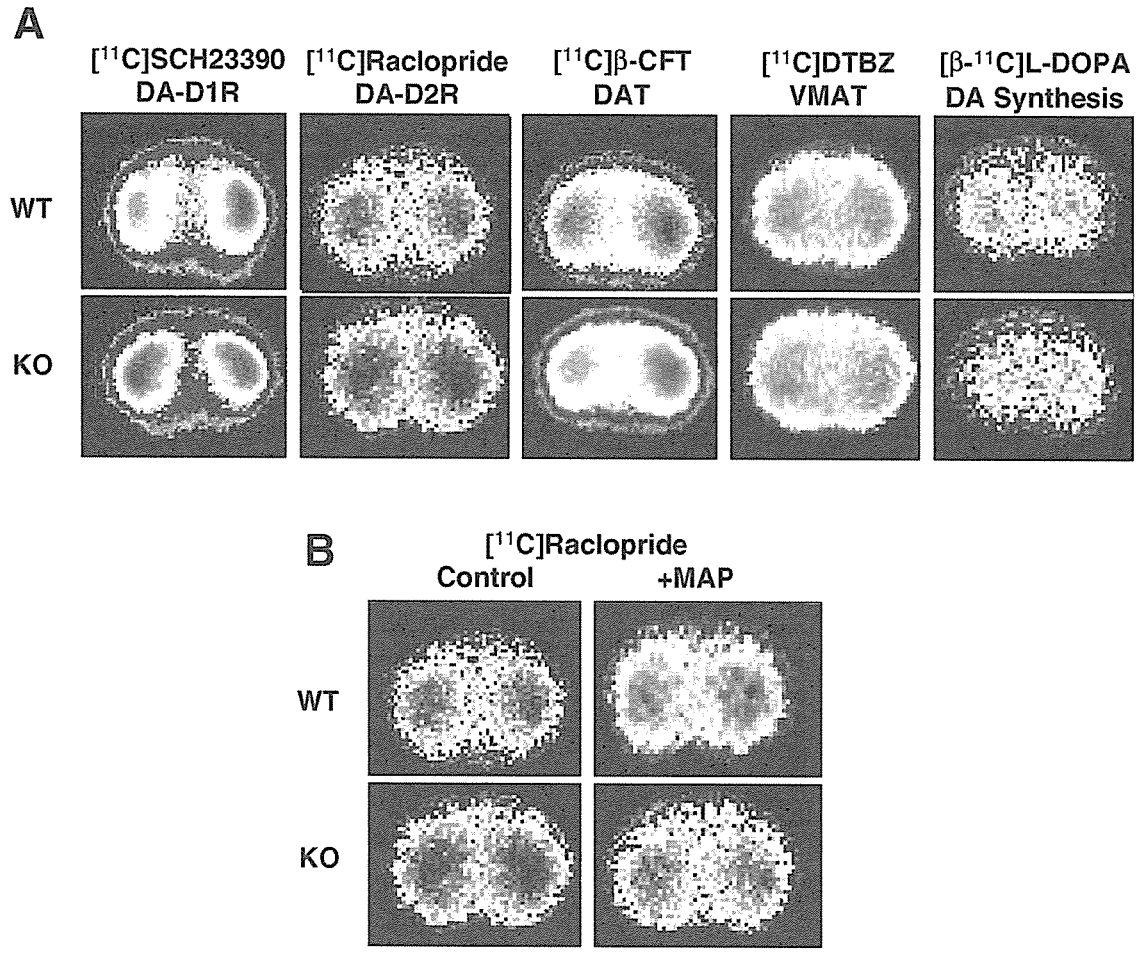


Fig. 5. Autoradiographic images. **A:** Comparison of striatal dopaminergic parameters of DA synthesis, DA receptors (D_1 and D_2), DAT, and VMAT between wild-type (WT) and parkin-deficient (KO) mice. **B:** Effects of methamphetamine (MAP) on striatal [^{11}C]raclopride binding in WT and KO mice. Saline or MAP at a dose of 0.3 mg/kg was injected i.v. 30 min before the injection of [^{11}C]raclopride. Brain slice preparation and imaging procedure were carried out as described in Materials and Methods.

uated these mice at 12 months of age using the rotorod task and found that *parkin*$^{-/-}$ (n = 10) and wild-type mice (n = 10) exhibited similar latencies for remaining on the rotating rod (Fig. 2), confirming the lack of any behavioral deficit. Furthermore, DAergic neurons of parkin-deficient mice were morphologically normal by immunohistochemical analysis with an antibody specific for TH (Fig. 3). Indeed, quantification of the number of DAergic neurons of the mutant and control mice (n = 6 each) at 3 months showed similar numbers of TH-positive neurons in SN and LC (data not shown).

Neurochemical Analysis

Next, we measured the levels of DA and its major metabolites DOPAC and HVA in *parkin*$^{-/-}$ and control mice (n = 10) at 12 months by HPLC with electrochemical detection. In the midbrain including SN, parkin-deficiency was associated with a significant increase in DA level but no change in DOPAC and HVA levels (Fig. 4). In the striatum, no changes in DA, DOPAC, and HVA levels were noted in *parkin*$^{-/-}$ mice compared to the wild-type.

Ex Vivo Autoradiography Study

Figure 5 shows representative autoradiographic images. The levels of two major DA receptors, D_1 and D_2, were measured at 12 months and expressed as the binding index by ex vivo autoradiography using receptor antagonists [^{11}C]SCH23390 and [^{11}C]raclopride, respectively. Quantitative analysis showed that the receptor binding levels of both D_1 and D_2 in the striatum of *parkin*$^{-/-}$ mice (D_1, n =7; D_2, n = 6) were higher than those of normal mice (D_1, n = 5; D_2, n = 6), although the binding index of dopamine transporter (DAT) and vesicular monoamine transporter (VMAT) using [^{11}C]β-CFT and [^{11}C]DTBZ, respectively, were similar (Fig. 6A)

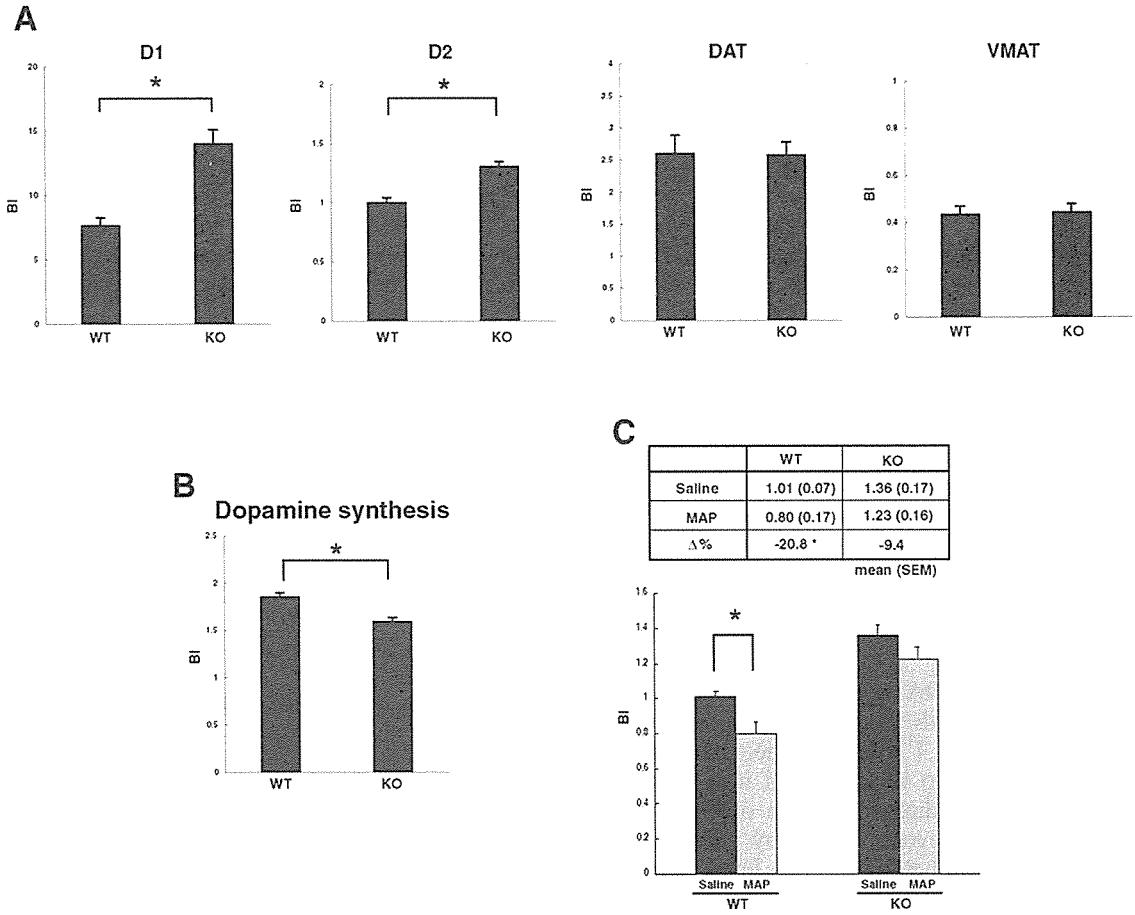


Fig. 6. Quantitative analysis of ex vivo autoradiography. **A:** Binding index for DA receptors (D_1 and D_2), DAT, and VMAT in the striatum of mice aged 12 months. **B:** DA synthesis in the striatum of mice aged 12 months. **C:** DA release of mice aged 12 months. Values of striatal [^{11}C]raclopride binding after administration of methamphetamine (MAP) or saline in wild-type and parkin-null mice are tabulated. %Δ, percentage difference [(methamphetamine binding index − saline binding index)/saline binding index] × 100. Data are expressed as mean ± SEM. *$P < 0.01$.

in *parkin*$^{-/-}$ mice (DAT, n = 7; VMAT, n = 6) and normal mice (DAT, n = 5; VMAT, n = 6). Furthermore, DA synthesis determined by conversion of L-[β-^{11}C]DOPA to [β-^{11}C]DA was significantly decreased in *parkin*$^{-/-}$ mice (n = 8) compared to control mice (n = 8) (Fig. 6B).

Finally, we analyzed DA release in the striatum of *parkin*$^{-/-}$ mice using dopamine D_2 receptor antagonist [^{11}C]raclopride after treatment of methamphetamine (MAP) or saline. [^{11}C]Raclopride is considered to compete with endogenous DA at D_2 receptor sites, and competition between [^{11}C]raclopride and endogenous DA has been used recently to measure the level of DA release (Tsukada et al., 2000; Piccini et al., 2003). The binding of [^{11}C]raclopride was decreased in the presence of high levels of released endogenous DA in the synaptic clefts. The release capacity of DA can be estimated by a decrease in the binding level after methamphetamine-induced release of endogenous DA. Ex vivo autoradiography conducted at 12 months using [^{11}C]raclopride showed that the activity of DA release in *parkin*$^{-/-}$ mice (n = 8) was clearly reduced relative to that in wild-type mice (n = 9) (Fig. 6C).

DISCUSSION

Several studies have reported deletion of the mouse *parkin* gene but against expectation, the majority of such mice exhibited subtle phenotypes without causing massive loss of DAergic neurons (Goldberg et al., 2003; Itier et al., 2003; Von Coelln et al., 2004; Perez and Palmiter, 2005). Our parkin-null mice showed no obvious behavioral deficit; testing mouse mobility by using the open field test (data not shown) and rotorod test (Fig. 2) showed no reduction in locomotor activity. Histologically, no change in TH-positive nigra neurons was noted in parkin-deficient mice (Fig. 3), and no significant decrease in DAT was observed (Fig. 6A). [^{11}C]β-CFT shows a high affinity to DAT and accurately reflects a terminal density close to that of DA neurons, which could be potentially useful in tracing the drop out of DA neurons over time. These results show clearly the lack of any neurodegeneration in the cell bodies of DA neurons and nerve terminals of parkin deficient mice.

In an attempt to determine the pathologic state of parkin-deficient mice, we assessed the binding of D_1 and D_2 receptors by ex vivo autoradiography. We used the [^{11}C]SCH23390 and [^{11}C]raclopride ligands against the D_1 and D_2 receptors, respectively. Intriguingly, parkin deficiency was associated with marked elevation of both D_1 and D_2 receptors. Indeed, previous studies reported that although striatal D_2 binding increased in untreated patients with PD, adaptive postsynaptic mechanisms and treatment decreased this as the condition advanced (Ahlskog et al., 1991; Brooks et al., 1992; Ichise et al., 1999). In a study of AR-JP patients, Scherfler et al. (2004) reported a global decrease in D_2 receptor and argued for parkin genetic defect itself or susceptibility to receptor downregulation after long-term exposure to dopaminergic agents. Taking into account the results of the present study, we consider the latter scenario; i.e., long-term exposure to dopaminergic agents, to result in downregulation of D_2 receptor. The decrease in DA concentration in synaptic clefts in early-stage PD is expected to lead to increased expression of D_2. Goldberg et al. (2003) reported that DA actually increases extracellularly in knockout mice.

We next measured DA release in knockout mice. When endogenous DA binds to the D_2 receptor, it competes with the antagonist [^{11}C]raclopride. This process allows synaptic DA levels to be estimated indirectly from changes in D_2 receptor binding. Elevation of DA synaptic concentrations can be achieved in vivo by administration of DA releaser such as amphetamine. The level of binding index in parkin-deficient mice was significantly higher than that of control mice, indicating a low DA release capacity in knockout mice (Fig. 6C). A similar method showed a decrease in release capacity of DA in PD (Piccini et al., 2003). However, this level of alteration of DA release potential is not sufficient to cause atrophy and degeneration of DAergic neurons, thus explaining the lack of a clear phenotype in knockout mice. Indeed, the open field and rotorod tests showed no reduction in locomotor activity, as mentioned above.

Quantitative analysis showed no differences in dopamine levels in the striatum of mutant and wild-type mice, although higher DA concentrations were noted in the midbrain of knockout mice relative to the control. This regional difference may be due to either accumulation of DA consequent to the low level of DA release, or due to increased DA reuptake. Although the expression levels of DAT and VMAT were not different between the two types of mice, further research is necessary to determine their precise functions. It is well-known that [^{18}F]DOPA undergoes reuptake in DAergic neurons and metabolizes to [^{18}F]DA, which is indicative of the synthetic capacity of DA involved in PD (Dagher, 2001; de la Fuente-Fernandez and Stoessl, 2002). Likewise, L-[β-^{11}C]DOPA is also metabolized to form [β-^{11}C]DA. Using this method, the overall synthetic capacity of DA was significantly low in knockout mice compared to wild-type mice (Fig. 6B). Excess DA in neurons may induce down-regulation of this synthetic capacity. In this regard, abnormal reuptake of [^{18}F]DOPA was reported previously in AR-JP patients (Broussolle et al., 2000; Hilker et al., 2001; Portman et al., 2001; Khan et al., 2002; Thobois et al., 2003; Scherfler et al., 2004). In contrast, the levels of DOPAC and HVA in the ventral midbrain were apparently normal in our knockout mice. This finding may be dependent on the regulation of activities of various enzymes that metabolize catecholamines, which compensate the altered DA transmission in parkin deficiency.

In conclusion, we have shown in the present study the presence of low levels of DA release in parkin-deficient mice, suggesting that DAergic neurons could behave abnormally before neuronal death.

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Leucine-Rich Repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population

Manabu Funayama[a], Yuanzhe Li[b], Hiroyuki Tomiyama[b], Hiroyo Yoshino[a], Yoko Imamichi[b], Mitsutoshi Yamamoto[c,f], Miho Murata[d,f], Tatsushi Toda[e,f], Yoshikuni Mizuno[a] and Nobutaka Hattori[a,b,f]

[a]Research Institute for Diseases of Old Age, [b]Department of Neurology, Juntendo University School of Medicine, Bunkyo-ku, Tokyo, [c]Department of Neurology, Kagawa Prefectural Central Hospital, Takamatsu, [d]Department of Neurology, Musashi Hospital, National Center of Neurology and Psychiatry, Tokyo, [e]Division of Clinical Genetics, Department of Medical Genetics, Osaka University Graduate School of Medicine, Suita, Osaka and [f]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan

Correspondence and requests for reprints to Dr/Professor Nobutaka Hattori, MD, PhD, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
Tel: +81 3 5802 1073; fax: +81 3 5800 0547; e-mail: nhattori@med.juntendo.ac.jp

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To assess the effect of genetic factors on sporadic Parkinson disease, we performed a case–control study of a variant (G2385R) in *Leucine-Rich Repeat kinase* 2 among the Japanese population. The G2385R (c.7153G>A) variant was reported as a risk factor for sporadic Parkinson disease in the Chinese population from Taiwan and Singapore. Genotyping was conducted in 448 Parkinson disease patients and 457 healthy controls. The frequency of A allele in Parkinson disease was significantly higher than in the control ($P=1.24\times10^{-4}$, odds ratio 2.63, 95% confidence interval 1.56–4.35). Our results suggest that the G2385R variant is a risk factor for sporadic Parkinson disease in the Asian population. *NeuroReport* 18:273–275 © 2007 Lippincott Williams & Wilkins.

Keywords: *Leucine-Rich Repeat kinase* 2, risk factor, single nucleotide polymorphisms

Introduction

Parkinson disease (PD) is one of the most frequent neurodegenerative diseases characterized by resting tremor, rigidity, bradykinesia, and postural instability. PD is thought to be a multifactorial disease caused by a combination of aging, environmental, and genetic factors. Although the majority of patients of PD are of sporadic type, some genes have been identified as a monogenic causative gene by molecular genetic studies for familial PD [1–6]. *Leucine-Rich Repeat kinase* 2 (*LRRK2*) has been identified as a causative gene associated with autosomal dominant familial PD [7,8]. To date, many pathogenic substitutions in *LRRK2* have been identified in familial and sporadic PD [9]. The G2385R variant (c.7153G>A) in *LRRK2* was reported recently as a risk factor for sporadic PD in the Chinese population from Taiwan and Singapore [10,11]. This variant was identified originally as putative pathogenic mutation in a small Taiwanese PD family and was not found in Caucasians [12]. Thus, it is possible that the G2385R variant is a risk factor in Asian sporadic PD. To test this hypothesis, we conducted a case–control study to evaluate the association between the G2385R genotype and the risk for PD in the Japanese population.

Methods

Subjects and genomic DNA

Genomic DNA was isolated from 448 sporadic PD patients and 457 controls of the Japanese population by a standard protocol (Table 1). All PD patients had no family history of PD. PD patients with *parkin* or *PTEN-induced putative kinase 1 (PINK1)* mutation were not included in the study. Diagnosis of PD was adopted by the participating neurologists and was established on the basis of the United Kingdom Parkinson's Disease Society Brain Bank criteria [13]. This study was approved by the ethics committee of Juntendo University School of Medicine. All individuals gave an informed and signed consent form.

Genotyping

Exon 48 of *LRRK2* from each individual was amplified by polymerase chain reaction (PCR) using the primers and protocol described by Zimprich *et al.* [8]. The PCR products were sequenced directly using the BigDye Terminators v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). The reverse PCR primer was used as sequencing primer.

Statistical analysis

Statistical analysis included the Hardy–Weinberg equilibrium test, χ^2 test, Fisher's exact test, odds ratio and its 95% confidence interval (95% CI), using SNPAlyze v5.1 software (Dynacom, Chiba, Japan). The *t*-test was performed using JMP 6.0 (SAS Institute Japan, Tokyo, Japan). In all statistical analyses, *P* values of 0.05 or less were considered statistically significant.

Results

We analyzed the frequency of the c.7153G>A (G2385R) substitution in 448 patients and 457 controls. Genotypes of the controls and patients were concordant with Hardy–Weinberg equilibrium. The frequency of A allele in the patients was significantly higher than in the controls ($P=1.24\times10^{-4}$, odds ratio 2.63, 95% CI 1.56–4.35, Table 2). We also detected homozygous substitution for the G2385R variant in two patients; however, we detected only the heterozygous substitution in the controls. Concerning the age at onset, the G2385R carriers were somewhat older than the noncarriers in total patients and in those <50 years of age. In contrast, the age at onset was not significantly different between carriers and noncarriers aged ≥50 years (Table 3). The disease duration was not significantly different between carriers and noncarriers (data not shown).

Discussion

In this study, we observed the *LRRK2* G2385R variant in 11.6% (52/448) of sporadic PD patients. So far, many putative pathogenic mutations have been reported including the G2385R. We detected G2385R in both patients and controls (22/457: 4.8%, Table 2); thus, this variant is not a pathogenic mutation, but a single nucleotide polymorphism. These results were similar to the allele frequencies in the Chinese [10,11]. It is estimated that mutations of *LRRK2* are the most frequent among the causative genes for autosomal dominant familial PD so far. Indeed, only one mutation (G2019S) accounted for ~6.6% of familial PD and ~1.6% of sporadic PD in Caucasians [14–16]. Interestingly, the frequency of the G2019S mutation is ~40% in the familial PD of North African Arabs [17] and ~30% in the familial PD of Ashkenazi Jews [18], whereas the G2019S mutation is a much less common mutation in Asians [19,20]. It is likely that some differences of genetic background exist among Caucasians, North African Arabs, Ashkenazi Jews, and Asians. Although G2385R has been detected only in Asian population, some risk variations in PD such as α-synuclein would be found in not only Asians but also all ethnic groups [21–24].

Among patients with age at onset <50 years, the G2385R carriers were somewhat older than noncarriers. This might indicate that G2385R has no influence on early-onset PD, and that PD of patients with early-onset might be influenced by other genetic and/or environmental factors. In addition, there were no differences in any clinical features including age at onset among carriers with homozygous or heterozygous G2385R substitution and noncarriers. Although the G2385R might increase the risk of development of PD, it does not seem to have a clear effect on modifying the symptoms or worsening the progression of the disease.

The amino-acid G2385 is located in the WD domain of LRRK2. This domain is known to bind various proteins [9]. The WD domain of LRRK2 appears to play an important role in neuronal cells. Indeed, oxidative-stress-induced cell death was more enhanced by the overexpression of G2385R variant than wild-type LRRK2 using culture cells [11]. More studies are needed to understand the functional significance of the substitution of glycine to arginine.

Conclusion

In this study, we identified that the G2385R variant in *LRRK2* is a risk for PD in Japanese population. To combine with the result of Chinese population [10,11], this variant increases the risk of PD in Asian population. So far, multiple genomic loci have been identified as susceptibility loci for PD [25], suggesting that many genes have a synergistic influence on the development of PD.

Table 1 Age characteristics of individuals

	Patients	Controls
Total sample, *n* (%)	448 (100)	457 (100)
Male, *n* (%)	217 (48.4)	240 (52.5)
Female, *n* (%)	231 (51.6)	217 (47.5)
Age at onset (years)[a]	50.7 ± 14.6 (5–89)	—
Male[a]	49.1 ± 14.8 (5–89)	—
Female[a]	52.2 ± 14.2 (7–82)	—
Age at sampling (years)[a]	59.4 ± 13.8 (15–93)	43.8 ± 16.0 (21–98)
Male[a]	57.8 ± 14.7 (15–93)	43.8 ± 14.5 (23–92)
Female[a]	60.9 ± 12.7 (22–88)	43.9 ± 17.5 (21–98)

[a]Data are mean ± SD (range).

Table 3 Comparison of age at onset of PD patients

Age at onset (years)	Carriers (*n*)	Noncarriers (*n*)	*P*-value
<50	42.5 ± 5.8 (17)	37.1 ± 9.4 (180)	0.003
≥50	59.9 ± 7.0 (33)	61.6 ± 7.8 (209)	0.24
Total	54.0 ± 10.6 (50)	50.3 ± 14.9 (389)	0.03

Data are mean ± SD.
Patients without information about age at onset (two of carriers and seven of noncarriers) were excluded from this analysis.
PD, Parkinson disease.

Table 2 Association analysis of *LRRK2* G2385R variant

	Genotype, *n* (%)			Allele, *n* (%)			
	G/G	G/A	A/A	G	A	χ^{2} [a]	*P*-value[a]
Patients (*n*=448)	396 (88.4)	50 (11.2)	2 (0.4)	842 (94.0)	54 (6.0)	14.74	1.24×10^{-4}
Controls (*n*=457)	435 (95.2)	22 (4.8)	0 (0)	892 (97.6)	22 (2.4)		

LRRK2, *Leucine-Rich Repeat kinase 2*.
[a]Compared with the control.

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Molecular mechanisms of nigral neurodegeneration in Park2 and regulation of parkin protein by other proteins

N. Hattori, Y. Machida, S. Sato, K. Noda, M. Iijima-Kitami, S. Kubo, and **Y. Mizuno**

Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

Summary. Most of the patients with Parkinson's disease (PD) are sporadic. However, Since identification of monogenic forms of PD, the contribution of genetic factors to the pathogenesis of sporadic PD is proposed as one of major risk factors. Indeed, this is supported by the demonstration of the high concordance in twins, increased risk among relatives of PD patients in case control and family studies. Thus, the functional analysis for the gene products for familial PD provides us a good hint to elucidate the pathogenesis of nigral degeneration. For example, although α-synuclein is involved in a rare dominant form of familial PD with dopa responsive parkinsonian features, this molecule is a major component of and Lewy bodies (LBs). In contrast, Park2 (parkin-related disease) is the most frequent form among patients with young-onset PD. However, Park2 brains generally lack the formation of LBs. In the other word, parkin responsible for Park2 is essential for the formation of LBs. Thus, both α-synuclein and parkin are speculated to share a common pathway. Here, we reviewed the parkin function and molecular mechanisms of Park2.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder primarily caused by selective dopaminergic cell loss in the midbrain substantia nigra pars compact. However, the exact cause of PD is still unknown. Since identification of monogenic form of PD, the functions of gene products for familial PD (FPD) have provided us good information for studying the mechanisms underlying neurodegeneration in PD. To date, eleven loci have been mapped and among them, six causative genes have been identified as causative genes in familial PD, which have significantly enhanced our understanding of the genetic mechanisms of not only FPD but also sporadic PD. Among the forms of FPD, the causative gene, *parkin* of AR-JP, representing the most prevalent form of familial PD (Kitada et al., 1998), is of a special interest, because it is liked to ubiquitin-proteasome system (UPS) as an E3 ubiquitin-protein ligase (Shimura et al., 2000), which covalently attaches ubiquitin to target proteins, designating them for degradation by the 26S proteasome (Pickart et al., 2001). These findings suggest that accumulation of the parkin substrate(s) due to loss-of-function of parkin induces loss of dopaminergic neurons. Thus, Park2 is caused by failure of proteolysis mediated by UPS (Dawson and Dawson, 2003). Since then, our knowledge of the substrate(s) for parkin has expanded, and indeed at present various putative substrates, such as

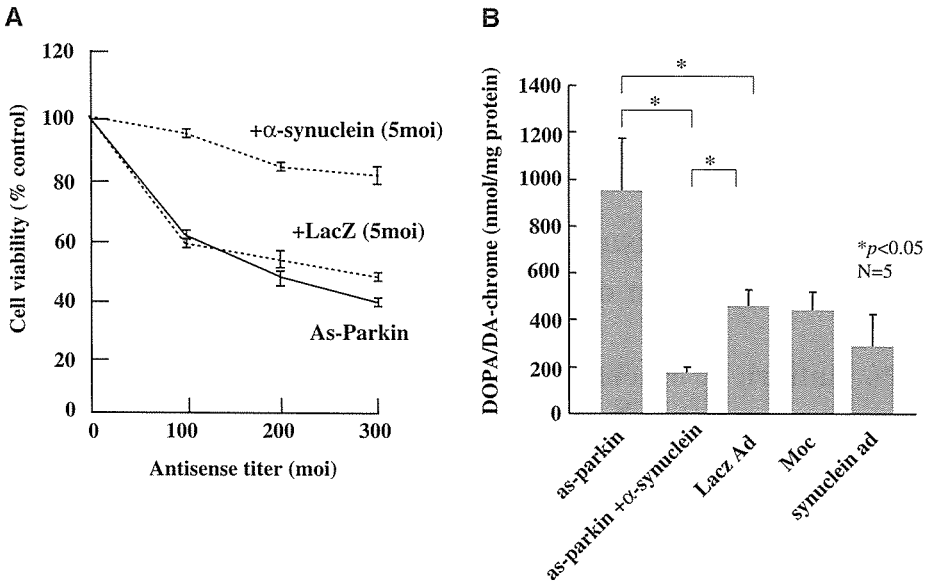

Fig. 1. α-Synuclein inhibits parkin knockdown-induced apoptosis and accumulation of DOPA- and DA-quinones. **A** Effects of overexpression of wild α-SN on as-parkin induced deterioration of cell viability. Differentiated SH-SY5Y cells were treated for 48 hours with as-parkin adenovirus. Cells were coinfected with LacZ and α-SN adenovirus (5 moi) and at the 150 moi titers of as-parkin adenovirus. The cell viability was measured and represented. **B** Cellular level of DOPA/DA-chromes. After the differentiated SH-SY5Y cells were treated for 36 hours with as-parkin, wild α-SN, LacZ and adenoviruses, cellular DOPA/DA-chromes were measured. Note the profound decrease of DOPA/DA-chromes in α-SN-expressing SH-SY5Y cells. Data are the mean ± SEM of 10 determinations. $^{*}P < 0.05$ versus control group (Tukey's multiple t test)

CDCrel-1, synphilin-1, α-SN22 (*O*-glycosylated form of α-SN), Pael-R etc, have been identified, but the pathophysiological role of parkin is still poorly understood (see review Hattori and Mizuno, 2004). Furthermore, null mice have no phenotypes for PD although several changes including dopamine metabolism have been reported so far. However, a direct link between these factors and dopaminergic cell death has not yet been reported. The important question of why dopaminergic neurons in the SN are particularly vulnerable to the loss-of-function effect of parkin remains to be determined. Although parkin is expressed ubiquitously in the brain, the pathologic findings of Park2 brains show severe neuronal loss with gliosis in the SN and mild neuronal loss in the locus coeruleus (LC), suggesting that the pathological change of Park2 brain is mainly in the SN. To investigate such selective neuronal loss, we established a good *in vitro* model by parkin knock down using full length antisense parkin.

Molecular mechanisms of Park2

Recently, two groups independently reported the generation of model mice lacking the *parkin* gene, which display certain abnormalities of dopamine metabolism (Itier et al., 2003; Goldberg et al., 2003). However, these parkin knockout mice had only subtle phenotypes exhibiting grossly normal brain morphology. In contrast, full-length human parkin antisense knocked-down endogenous parkin protein in differentiated human neuroblastoma cells (SH-SY5Y), 12 to 36 hours after infection and reduced cell viability (Machida et al., 2005). In addition, control β-galactosidase expressing adenoviruses could not knockdown

parkin and failed to affect cell viability. Thus, this system itself using adeno virus is not cytotoxic to the culture cells. The specificity of the antisense effect was confirmed by the result of co-infection of sense *parkin* expressing adenovirus, which abrogated reduction of cell viability. On the other hand, *parkin* antisense had no effect on cell viability of HeLa cells, suggesting that *parkin* antisense exert a specific effect on the cell viability of differentiated SH-SY5Y cells. Thus, this *in vitro* model is a powerful tool for elucidating the several issues as mentioned before.

Although *in vitro* system could induce the cell loss, why do parkin knockout mice lack abnormalities like AR-JP patients? One plausible explanation is the presence of a putative molecule(s) that suppresses the defect induced by loss-of-function of parkin, and such molecule(s) present abundantly in the brain should be linked to the pathogenesis of PD. Here we propose that α-SN is the molecule that compensates for the loss of parkin, since α-SN prevented apoptotic cell death induced by as-parkin. In this regard, Western blotting analysis showed that the dopaminergic SH-SY5Y cells did not express α-SN at significant levels, which is in marked contrast to the high abundance of dopaminergic neurons *in vivo*. Regardless of the compensating role of α-SN for the loss-of-function of parkin in Park2, α-SN is probably unable to cope with the accumulation of toxic molecules in the absence of parkin and thus apoptotic neuronal death perhaps occurs gradually, leading to degeneration of dopaminergic neurons. This is the first evidence for the anti-apoptotic role of α-SN and its involvement in the common pathway of parkin.

To date, several studies have demonstrated that α-SN could exert protective effect against various cellular stresses such as oxidative damage and related apoptosis of neurons. Considering the reason why mutation of α-SN causes familial PD, it is clear that this type of disease is due to the gain-of-toxic function of the mutant α-SN, differing from neuroprotective roles of the wild-type α-SN. In this context, it is noteworthy that α-SN is a major component of Lewy bodies, the pathological hallmark of PD, and at high concentrations, it oligomerizes to β-pleated sheets known as protofibrils (i.e., fibrillar polymers with amyloid-like characteristics). In addition, α-SN proteins with disease-causing mutations tend to generate protofibrils, suggesting that protein misfolding including α-SN plays a key role in the pathogenesis of PD. In addition to our finding, α-SN could play dual function such as neuroprotection and or neurotoxicity. Considering the presence of the patients with α-SN multiplication, overproduction of this molecule could cause for developing PD, and lower level expression would be also cytotoxic to the dopaminergic neurons. Indeed, α-SN knock out mice displayed the impairment of the dopamine release although neuronal loss has not been reported so far.

It remains unclear why dopaminergic neurons of the substantia nigra and locus coeruleus are selectively vulnerable to the loss of parkin in AR-JP patients. In the present study, we provided a clue for this enigmatic puzzle. Considering the specificity of the lesions in PD, it is possible that the high oxidative state associated with DA metabolism may cause deterioration of dopaminergic neurons. The mechanism underlying increased oxidative stress may involve DA itself, because oxidation of cytosolic DOPA/DA may be deleterious to neurons. Indeed, DA causes apoptotic cell death with morphological nuclear changes and DNA fragmentation. In this regard, we showed here that as-parkin directed loss of parkin leads to abnormality of DOPA/DA metabolism, which generated DOPA/DA-quinones in SHSY5Y cells. Thus, DA and its metabolites seem to exert cytotoxicity mainly by generating highly reactive quinones through auto-

oxidation. On the other hand, the toxicity of DOPA and DA is due to the generation of reactive oxygen species that could disrupt cellular integrity, causing cell death. However, the reason for the production of oxidative DOPA/DA-metabolites following loss of parkin is not clear at present.

The loss-of-function of parkin by full length antisense strategy could lead to the cell death of differentiated dopaminergic cells *in vitro*. In addition, the increasing of DOPA/DA-quinones was associated with the cell death, suggesting that quinines derived from dopamine metabolism are killer molecules in Park2 brains. This cell-based experiment enhances our understanding of the pathophysiology of PD and is potentially useful for drug screening in the future. Our results also showed that α-SN and parkin are involved in DA metabolism and its aberrant regulation is accompanied by accumulation of oxidative DOPA/DA metabolites.

Recently, parkin has been negatively regulated by S-nitrosylation modification and BAG5 (Kalia et al., 2004; Chung et al., 2004). Thus, loss-of-function through parkin mutation as in Park2, nitrosylation or binding with BAG5 results in nigral degeneration in not only Park2 brains but also sporadic PD. In the other word, such negative regulation system for parkin ubiquitin ligase suggests a possible mechanistic link between the familial and sporadic forms of PD. As s-nytrosylation for parkin has been reported in sporadic PD, DOPA/DA metabolites could be also involved in the pathogenesis for sporadic form of PD as well as Park2 brains. Finally, several gene products have been reported so far, the relationship among them is unclear at present. However, considering clinical similarities including neuropathologic findings between sporadic and FPD, most of the gene products may share a common pathway on the pathogenesis for PD.

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Author's address: N. Hattori, Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo, Tokyo 113-8421, Japan, e-mail: nhattori@med.juntendo.ac.jp

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Progress in familial Parkinson's disease

Y. Mizuno, N. Hattori, H. Yoshino, Y. Hatano, K. Satoh, H. Tomiyama, and **Y. Li**

Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan

Summary. To date 11 forms of familial Parkinson's disease (PD) have been mapped to different chromosome loci, of which 6 genes have been identified as the causative genes, i.e., *alpha-synuclein* (*SNCA*), *parkin*, *UCH-L1*, *PINK1*, *DJ-1*, and *LRRK2*. For *UCH-L1*, additional families with this mutation are necessary before concluding that *UCH-L1* is the definite causative gene for PARK5, as only one family so far has been reported. *SNCA*, *UCH-L1*, and *LRRK2* mutations cause autosomal dominant PD and the remaining gene mutations autosomal recessive PD. Age of onset tends to be younger in familial PD compared with sporadic PD, particularly so in autosomal recessive PD. Generally familial cases respond to levodopa quite nicely and progression of the disease tends to be slower. It is an interesting question how familial PD-causing proteins are mutually related each other. In this article, we review recent progress in genetics and molecular biology of familial PD.

Introduction

To date 11 forms of familial Parkinson's disease (PD) have been mapped to different chromosome loci (Table 1). In this article we review recent progress in these familial forms of PD.

PARK1

PARK1 is an autosomal dominant familial PD caused by mutations of *alpha-synuclein* (*SNCA*). Clinical features of PARK1 were first described by Golbe et al. (1990) on large autosomal dominant kindreds immigrated to USA from Contursi, a village in the hills of Salerno Province in southern Italy. Ancestors of this family are believed to have moved to Italy from Greece. Clinical features consist of L-dopa-responsive parkinsonism and variable degrees of cognitive impairment. The average age of onset of the original families reported by Golbe et al. (1990) was 46.5 ± 10.8 years (range, 28–68, $N = 33$).

Alpha-synuclein has been mapped to the long arm of chromosome 4 at 4q21-q23. To date, 3 missense mutations, i.e., A30P (Krüger et al., 1998), E46K (Zarranz et al., 2004), and A53T (Polymeropoulos et al., 1997) and triplication (Singleton et al., 2003) and duplication (Chartier-Harlin et al., 2004; Ibenez et al., 2004) of the entire *alpha-synuclein* are known (Fig. 1). Alpha-synuclein is a neuron-specific protein localized mainly in the presynaptic terminal membranes and synaptic vesicles. Although the function of alpha-synuclein is not well known, aggregated alpha-synuclein is accumulated in the nigral neurons in PD indicating that alpha-synuclein plays an important role in the pathogenesis of PD. Recently reported families with triplication and duplication of *alpha-synuclein* suggest that overexpression of normal alpha-synuclein per se is neurotoxic to nigral neurons.

Table 1. Inherited forms of Parkinson's disease

Name	Inheritance	Locus	Gene
PARK1	AD	4q21-23	*α-synuclein (SNCA)*
PARK2	AR	6q25.2-27	*parkin*
PARK3	AD	2p13	*unknown*
PARK4	AD	4q21-23	*α-synuclein*
PARK5	AD	4p14	*UCH-L1*
PARK6	AR	1p35-36	*PINK1*
PARK7	AR	1p36	*DJ-1*
PARK8	AD	12p11.2-q13.1	*LRRK2/ dardarin*
PARK9	AR	1p36	*unknown*
PARK10	AD/AR/SP	1p32	*unknown*
PARK11	AD	2q36-37	*unknown*

AD autosomal dominant, *AR* autosomal recessive, *SP* sporadic

Regarding the relationship between the types of mutation and clinical features, triplication and E46K mutations are associated with dementia in addition to parkinsonism and wide-spread neuropathologic changes with cortical Lewy bodies in addition to nigral neurodegeneration (Farrer et al., 1999; Zarranz et al., 2004). Actually neuropathological characteristics are consistent with those of diffuse Lewy body disease. On the other hand, duplication was associated with pure L-dopa-responsive parkinsonism without dementia. Ala53Thr mutation is associated with variable degrees of cognitive impairment. Ala30Pro is less likely to show cognitive impairment.

Functions of alpha-synuclein are not well known. Alpha-synuclein is a natively unfolded brain specific protein consisting of 140 amino acids without significant amount of secondary structure (Weinreb et al., 1996). From its localization in presynaptic terminals, it has been speculated that it may be related to neurotransmitter regulation. Alpha-synuclein has a tendency for self-aggregation and oligomer formation. Soluble oligomers ultimately form insoluble aggregates, which are the major component of Lewy bodies (Spillantini et al., 1998). Particularly, oligomers of alpha-synuclein are toxic to neurons inducing release of dopamine into the cytoplasm from synaptic vesicles (Volles and Lansbury, 2002), impairment of 26S proteasome (Snyder et al., 2003), and mitochondrial dysfunctions (Tanaka et al., 2001). Mitochondrial impairment results in reduced ATP synthesis. As 26S proteasome is an ATP-dependent protein degrading enzyme, mitochondrial impairment reduces its catalytic activity. Thus vicious cycles are formed within nigral neurons leading them to slowly progressing neuronal death. Mutated alpha-synuclein proteins show increased tendency for self-aggregation (El Agnuf et al., 1998).

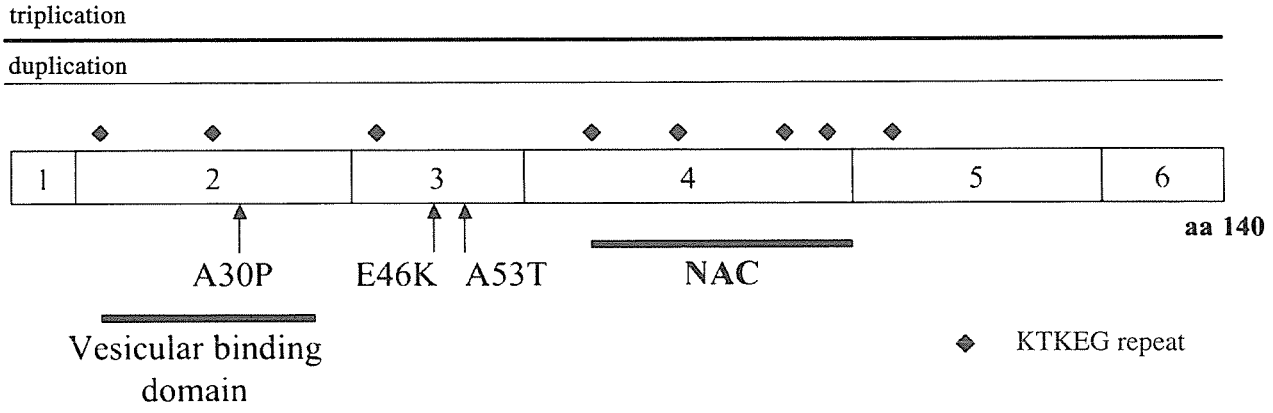

Fig. 1. Schematic presentation of the exons of *alpha-synuclein* and its mutations. Three missense mutations, duplication (thin line) and triplication (thick line) are known. Closed diamonds indicate approximate positions of the KTKEGV repeats. NAC represents non-amyloid component of the senile plaque

This is likely to be a reason for earlier onset of ages in familial PD due to alpha-synuclein mutations compared with sporadic PD. Furthermore, aggregated insoluble alpha-synuclein proteins are likely to impair transport of vital substances within nigral neurons. The insoluble aggregate of alpha-synuclein is highly phosphorylated at Ser-129 (Fujiwara et al., 2002).

PARK2

PARK2 is an autosomal recessive familial PD caused by mutations of *parkin*. Clinical features of PARK2 were first described by Yamamura et al. (1973). They reported 16 patients (13 familial patients in 5 unrelated families and 3 sporadic cases); clinical features of 11 patients from the initial 4 families were essentially identical. Ages of onset were between 17 and 28 years in 10 out of 11 patients and 42 in the remaining one. All the patients showed tremor, rigidity, bradykinesia, and postural instability. Atypical features included sleep benefit, temporary improvement of parkinsonism after a nap or sleep, and dystonic postures in the feet during walking (talipes equinovarus). Dementia was absent.

These patients show good response to L-dopa but they frequently develop motor fluctuations (wearing off and dyskinesia) sooner than late onset PD patients; usually two to three years after the initiation of L-dopa. Since the gene analysis became possible, many atypical features have been reported. For instance, age of onset can be as late as 72 (Lincolon et al., 2003). Other atypical features reported include dementia (Benbuman et al., 2004), psychosis and behavioral problem (Kahn et al., 2003), cerebellar ataxia (Kuroda et al., 2001), peripheral neuropathy (Tassin et al., 1998; Okuma et al., 2003; Kahn et al., 2003), hyperhidrosis (Yamamura et al., 1998), orthostatic hypotension (Kahn et al., 2003), urinary urgency and impotence (Kahn et al., 2003), and hemiparkinsonism-

Fig. 2. Schematic presentation of exons of parkin and its exon rearrangements. summarized from the following literature, i.e., Hattori et al. (1998), Abbas et al. (1999), Lücking et al. (2000), Oliviera et al. (2003), and Hedrick et al. (2004). Broken lines indicate triplication, dotted lines duplication, and solid lines deletions of exons. *UBL* ubiquitin-like domain, *RING* RING domain, *IBR* in-between RINGs

hemiatrophy (Pramistaller et al., 2002) have been reported.

PARK2 is caused by mutations of *parkin* (Kitada et al., 1998), which has been mapped to the long arm of chromosome 6 at 6q25.2-q27. To date more than 30 different exon rearrangements (deletion, duplication, and triplication) (Fig. 2), 30 missense mutations, and 8 nonsense mutations, and close to 20 small deletions or insertions (Fig. 3) have been reported (Hattori et al., 1998; Abbas et al., 1999; Lücking et al., 2000; Oliviera et al., 2003; Hedrick et al., 2004). These numbers are still increasing. Usually PARK2 patients harbor either homozygous mutations or compound heterozygous mutations of *parkin*. But at times single heterozygous mutations are seen. According to our hand, approximately 20% of patients with *parkin* mutations were single heterozygotes, in that only one allele of *parkin* showed a mutation and we could not find the second mutation. Question is how they could have got the disease. As PARK2 is an autosomal recessive disorder, it is expected that both of parkin alleles are mutated. Although exact mechanism is not known, numbers of possibilities can be considered. For instance, single normal parkin may not be suffice to its complete function (haploinsufficiency); mutated parkin protein in some way may interfere with the function of normal parkin protein (dominant-negative effect); single mutated parkin may predispose to late onset Lewy body-positive PD.

Parkin protein was found to be an ubiquitin-protein ligase (E3) of the ubiquitin system (Shimura et al., 2000). The ubiquitin-proteasome system (UPS) is an important intracellular proteolytic system responsible for wide variety of biologically important cellular processes, such as cell-cycle progression, signaling cascades, developmental programs, the protein quality control system, DNA repair, apoptosis, signal transduction, transcription,

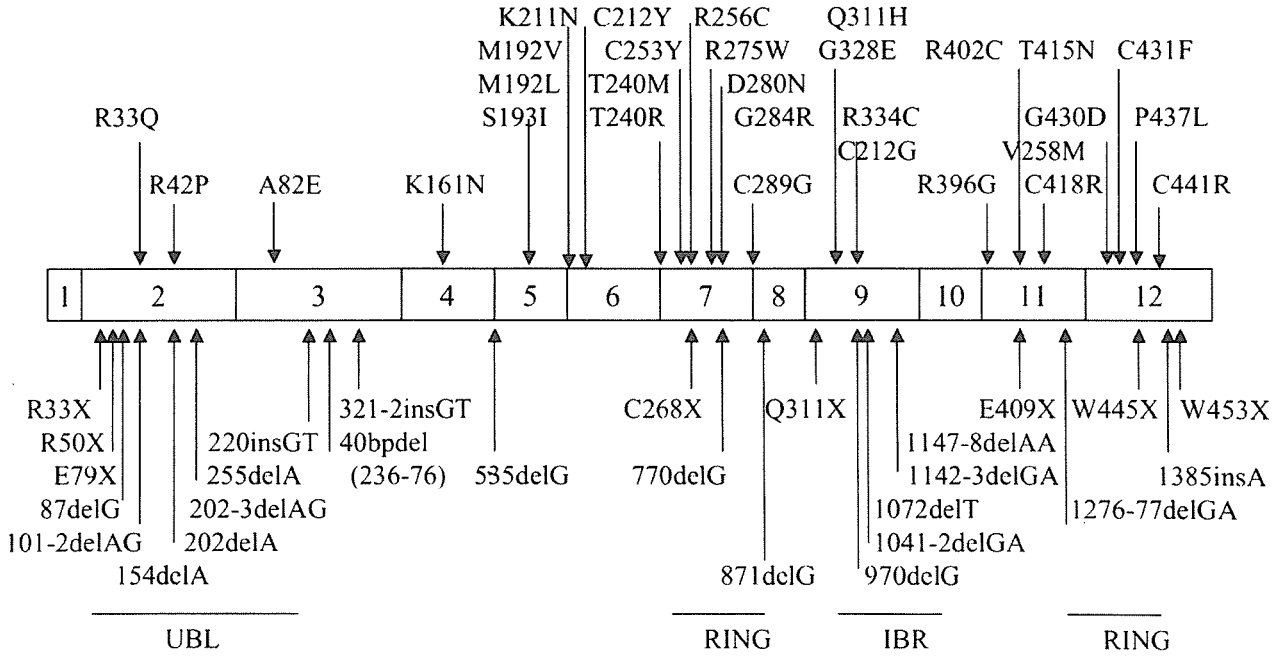

Fig. 3. Schematic presentation of exons of parkin and missense mutations, nonsense mutations, and small deletions summarized from the following literature, i.e., Hattori et al. (1998), Abbas et al. (1999), Lücking et al. (2000), Oliviera et al. (2003), and Hedrick et al. (2004). Missense mutations are shown above the exons and nonsense mutations and small deletions below the exons. *UBL* ubiquitin-like domain, *RING* RING domain, *IBR* in-between RINGs